



Important rat models of human diseases include those for cardiovascular diseases, neurodegenerative diseases, behavioral disorders, metabolic disorders, and carcinogenesis. Mammalian cloning may enhance these models.

The “New” Genetics and Mammalian Cloning in Environmental Health Research

Recent advances in genetic technology have spurred a mini-revolution in the study of toxicology. Toxicologic studies are a national imperative, and the importance of the application of transgenic mice and knock-out technologies to these studies is widely recognized. For example, the use of Tg.AC transgenic mice, carrying an inducible *v-H-ras* gene, and *p53*^{+/−} mice speeds the outcomes of the traditional 2-year bioassay of chemicals nominated for study (1–8). Mechanistic studies have been greatly enhanced by Big Blue transgenic animals that allow “shuttle” mutagenesis studies (9–11).

These genetic approaches have enhanced our knowledge of mechanisms that are important to molecular toxicology as well. By knocking out gamma-glutamyl transpeptidase, the paradoxical reduction of intracellular glutathione was found to be associated with the accumulation of DNA damage (12). Mechanistic roles for repair enzyme genes in toxicologic damage have been revealed with this technology. For example, mouse models of xeroderma pigmentosa produced by creating null mutations of *xpc* gene prove the critical function nucleotide excision repair by the *xpc* system in ultraviolet radiation-induced damage leading to skin cancer (13). By combining mutations, the overlapping roles of *p53* (Trp53) and *xpc*, as well as base excision repair and mismatch repair, were revealed (14).

Similarly, this approach established the role of β -pol in long patch repair and established that the failure of this repair system can lead to chromosomal breakage and apoptosis (15,16). β -pol null cells were used to show that removal of 5'-deoxyribose phosphate moiety from DNA is a key step in base excision repair (17). The promise now is that knock-out technology, particularly combined with widespread application of gene array studies, will enhance the Environmental Genome Project goal of establishing mechanisms of gene-environment interaction (18).

The application of these technologies through model systems (fruitfly and *Caenorhabditis elegans*) that establish “the usual suspect” genes by sequence similarities was recently boosted with the completion of both the *Drosophila* and *C. elegans* genome projects (19). These projects revealed a surprising level of sequence conservation to the human. In the case of *Drosophila*, sequence homology to humans is estimated to be approximately 50%, and > 60% of a subset of human disease genes (68% of human cancer genes) had orthologs in the *Drosophila* annotated genome. We know this conservation extends to important aspects of complete pathways as well, such as the Sonic hedgehog–Patched–GLI pathway (20).

The ability to take information from the model system to functional gene study with gain of function (e.g., transgenic) and loss of function (e.g., knock-out) mutations in analogous experimental systems such as the mouse is extremely powerful because of the genetic information available in mouse strains. It is important to remember that complete exploitation of this approach requires careful phenotypic analysis, which is often not available or difficult to obtain in the mouse.

Much of these data are already available or easily obtainable in the rat, however. Using the rat, physiologic and pathophysiologic data for common diseases and metabolic pathways have been gathered for nearly a century from models of diseases that are important to the



national public health. Often the rat model most closely resembles the human from among acceptable experimental systems. Important rat models of human diseases include those for cardiovascular diseases, neurodegenerative diseases, behavioral disorders, metabolic disorders, and carcinogenesis, all of which have important environmental overlays that are often poorly understood at the mechanistic level (21,22).

The genomic resources for using rat models of human disease conditions are robust and growing rapidly (23,24). Particularly important in this regard is the recent announcement that the rat genome will be sequenced. Currently, over 97% of the rat genome is covered at high density by anonymous markers, and the rat expressed sequence tag project has about 60,000 National Center for Biotechnology Information (Bethesda, MD) UniGene clusters. Polymorphisms in genes relevant to human toxicologic exposure have been studied in the rat for many decades. Recently, there have been several national initiatives to establish centers to maintain and distribute important rat strains of known genetic and microbiologic quality, and these should be available to environmental health scientists in the next few years. However, the full impact of these resources on mechanistic genetic studies is currently limited by the inability to produce knock-out rats. Indeed, at the time of this writing, knock-outs have only been successfully produced in the mouse.

This limitation may be overcome by establishing methods for nuclear transfer or cloning for the rat (25). Nuclear transfer is the process of removing the nuclear chromosomal material from unfertilized oocytes and replacing it with a nucleus from another cell, often an adult cultured cell (26). The nucleus reprograms, presumably by resetting methylated gene imprints, and the nuclear transfer oocyte can develop to term (27). The offspring from this process carries the genetic traits (including targeted mutations if present) of the cultured cells. Knock-out mice are currently produced by growing embryo-derived pluripotent stem cells in culture, creating targeted mutations in them and then transplanting them into preimplantation mouse embryos to produce a chimera that comprises normal recipient lineages as well as lineages derived from the mutant cells. If the chimera has functional germ cells that are derived from the targeted cells, then the mutation can be bred from the chimeras to produce heterozygous or homozygous mutant mice. As mentioned previously, this does not work in other animals because stem cells capable of producing germ line chimeras have not, so far, been reproducibly isolated except from certain strains of mice. On the other hand, nuclear transfer has been successful in producing clones of animals from several mammalian species (28–32), and results with the rat to date are encouraging. At this point it is possible to obtain advanced preimplantation stages from nuclear transfer rat oocytes, which is a prerequisite to developing animals. We have transfected embryonic fibroblasts with green fluorescent protein marker transgenes, and after selection, we used the cells as nuclear donors for nuclear transfer experiments. The genetically

modified rat cells are as efficient nuclear donors as unmodified cells are, so this method of making knock-out rats should work.

There are problems with this approach, however. The efficiency of producing cloned mammals appears to be approximately 2% of manipulated embryos, a number that seems not to be affected by the species used or the type of cell used as a nuclear donor. Currently, large-scale efforts to improve this efficiency are under way for several species of mammals. There is a high frequency of gestational loss of nuclear transfer fetuses with a wide variety of abnormalities, including high frequencies of placental abnormalities, regardless of the species used. The biochemical reprogramming required by the nucleus after transfer does not occur in a completely successful manner for the vast majority of embryos. Just what effect this would have on the phenotypic analysis of traits in animals produced this way is unclear. Nevertheless, making lines of animals with specific genetic traits in this way seems entirely feasible.

There are some very interesting questions that cloning raises independent of their potential utility in mechanistic genetic toxicology: What is nuclear reprogramming? What is the age of the animals relative to the age of the cells used as nuclear donors? What is the nature of the abnormalities in some of the clones? Why is the efficiency of cloning low and seemingly invariant? How do imprinted genes behave in cloned animals? What is the mechanism of methylation maintenance in cloned animals? Regardless of these questions and challenges, nuclear transfer offers the promise of providing a route to loss of function mutations in the rat. Nuclear transfer may provide the opportunity to exploit the marriage of phenotypic data, genomic data, expressed-gene data, and sequence conservation in pursuit of the goal of complete functional analysis of genes that modulate environmental exposure in human health. It is an exciting time for developing embryologic approaches to toxicology.

Philip M. Iannaccone

Department of Pediatrics

Northwestern University Medical School

The Children's Memorial Institute for Education and Research

Chicago, Illinois

E-mail: pmi@nwu.edu

REFERENCES

- Stoll RE, Holden HE, Barthel CH, Blanchard KT. Oxymetholone: III. Evaluation in the p53⁺- transgenic mouse model. *Toxicol Pathol* 27:513-518 (1999).
- Holden HE, Stoll RE, Blanchard KT. Oxymetholone: II. Evaluation in the Tg-AC transgenic mouse model for detection of carcinogens. *Toxicol Pathol* 27:507-512 (1999).
- Holden HE, Studwell D, Majeska JB. Oxymetholone: I. Evaluation in a comprehensive battery of genetic toxicology and in vitro transformation assays. *Toxicol Pathol* 27:501-506 (1999).
- Spalding JW, French JE, Tice RR, Furedi-Machacek M, Haseman JK, Tennant RW. Development of a transgenic mouse model for carcinogenesis bioassays: evaluation of chemically induced skin tumors in Tg.AC mice. *Toxicol Sci* 49:241-254 (1999).
- Tennant RW, Tice RR, Spalding JW. The transgenic Tg.AC mouse model for identification of chemical carcinogens. *Toxicol Lett* 102-103:465-471 (1998).
- Bucher JR, Lucier G. Current approaches toward chemical mixture studies at the National Institute of Environmental Health Sciences and the U.S. National Toxicology Program. *Environ Health Perspect* 106(suppl 6):1295-1298 (1998).
- Bucher JR. Update on National Toxicology Program (NTP) assays with genetically altered or "transgenic" mice. *Environ Health Perspect* 106:619-621 (1998).
- Mahler JF, Flagler ND, Malarkey DE, Mann PC, Haseman JK, Eastin W. Spontaneous and chemically induced proliferative lesions in Tg.AC transgenic and p53-heterozygous mice. *Toxicol Pathol* 26:501-511 (1998).
- Schmezer P, Eckert C. Induction of mutations in transgenic animal models: BigBlue and Muta Mouse. *IARC Sci Publ* 146:367-394 (1999).
- Mayer C, Klein RG, Wesch H, Schmezer P. Nickel subsulfide is genotoxic in vitro but shows no mutagenic potential in respiratory tract tissues of BigBlue rats and Muta Mouse mice in vivo after inhalation. *Mutat Res* 420:85-98 (1998).
- Cosentino L, Heddle JA. A test for neutrality of mutations of the lacZ transgene. *Environ Mol Mutagen* 28:313-316 (1996).
- Rojas E, Valverde M, Kala SV, Kala G, Lieberman MW. Accumulation of DNA damage in the organs of mice deficient in gamma-glutamyltranspeptidase. *Mutat Res* 447:305-316 (2000).
- Cheo DL, Meira LB, Hammer RE, Burns DK, Doughty AT, Friedberg EC. Synergistic interactions between XPC and p53 mutations in double-mutant mice: neural tube abnormalities and accelerated UV radiation-induced skin cancer. *Curr Biol* 6:1691-1694 (1996).
- Friedberg EC, Bond JP, Burns DK, Cheo DL, Greenblatt MS, Meira LB, Nahari D, Reis AM. Defective nucleotide excision repair in xpc mutant mice and its association with cancer predisposition. *Mutat Res* 459:99-108 (2000).
- Dianov GL, Prasad R, Wilson SH, Bohr VA. Role of DNA polymerase beta in the excision step of long patch mammalian base excision repair. *J Biol Chem* 274:13741-13743 (1999).
- Ochs K, Sobol RW, Wilson SH, Kaina B. Cells deficient in DNA polymerase beta are hypersensitive to alkylating agent-induced apoptosis and chromosomal breakage. *Cancer Res* 59:1544-1551 (1999).
- Sobol RW, Prasad R, Evenski A, Baker A, Yang XP, Horton JK, Wilson SH. The lyase activity of the DNA repair protein beta-polymerase protects from DNA-damage-induced cytotoxicity. *Nature* 405:807-810 (2000).
- Afshari CA, Nuwaysir EF, Barrett JC. Application of complementary DNA microarray technology to carcinogen identification, toxicology, and drug safety evaluation. *Cancer Res* 59:4759-4760 (1999).
- Rubin GM, Yandell MD, Wortman JR, Gabor Miklos GL, Nelson CR, Hariharan IK, Fortini ME, Li PW, Apweiler R, Fleischmann W, et al. Comparative genomics of the eukaryotes. *Science* 287:2204-2215 (2000).
- Walterhouse DO, Yoon JW, Iannaccone PM. Developmental pathways: Sonic hedgehog-Patched-Gli. *Environ Health Perspect* 107:167-171 (1999).
- Report of the NIH Rat Model Priority Meeting, 3 May 1999. Available: <http://www.nhlbi.nih.gov/resources/docs/ratmtgpg.htm> [cited 1 August 2000].
- Report of the NIH Rat Model Repository Workshop, 19-20 August 1998. Available: <http://www.nhlbi.nih.gov/meetings/model/index.htm> [cited 1 August 2000].
- Steen RG, Kwitek-Black AE, Glenn C, Gullings-Handley J, Van Etten W, Atkinson OS, Appel D, Twigger S, Muir M, Mull T, et al. A high-density integrated genetic linkage and radiation hybrid map of the laboratory rat. *Genome Res* 9:AP1-8, insert (1999).
- Jacob HJ. Functional genomics and rat models. *Genome Res* 9:1013-1016 (1999).
- Fitch P, Taborn G, Garton R, Iannaccone P. Nuclear transfer in the rat: potential access to the germline. *Transplant Proc* 31:1525-1530 (1999).
- Prather RS, First NL. Nuclear transfer in mammalian embryos. *Int Rev Cytol* 120:169-190 (1990).
- Wakayama T, Tateno H, Mombaerts P, Yanagimachi R. Nuclear transfer into mouse zygotes. *Nat Genet* 24:108-109 (2000).
- Wakayama T, Perry ACF, Zuccotti M, Johnson KR, Yanagimachi R. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* 394:369-374 (1998).
- Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KHS. Viable offspring derived from fetal and adult cells. *Nature* 385:810-813 (1997).
- Eyestone WH, Campbell KH. Nuclear transfer from somatic cells: applications in farm animal species. *J Reprod Fertil Suppl* 54:489-497 (1999).
- Wells DN, Misica PM, Tervit HR. Production of cloned calves following nuclear transfer with cultured adult mural granulosa cells. *Biol Reprod* 60:996-1005 (1999).
- Wolf E, Zakhartchenko V, Brem G. Nuclear transfer in mammals: recent developments and future perspectives. *J Biotechnol* 65:99-110 (1998).



Visit EHP Career Opportunities online

- updated weekly
- most ads include e-mail links for instant response

Just point and click.